GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

# PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ____

Project No. G-33-W04 (R-5983-4A0) GTRC/~~GIT~~ DATE 7 /11 /85

Project Director: S. W. May School/~~Lab~~ Chem.

Sponsor: DHHS/PHS/NIH/NHLBI

Type Agreement: Grant 2R01-HL28167-04

Award Period: From 7/1/85 To 6/30/86 (Performance) 9/30/86 (Reports)

| Sponsor Amount: | This Change | Total to Date |
|---|---|---|
| Estimated: | $ | $ 196,489 |
| Funded: | $ | $ 196,489 |

Cost Sharing Amount: $ 10,343 Cost Sharing No: G-33-390

Title: Novel Antihypertensives: Rational Design and Evaluation

ADMINISTRATIVE DATA OCA Contact John Schonk x4820

1) Sponsor Technical Contact:

Armando Sandoval
National Institutes of Health
National Heart, Blook, and Lung Inst.
Bethesda, MD 20205

301/496-1857

2) Sponsor Admin/Contractual Matters:

Willis A. Trawick
National Institutes of Health
National Heart, Blood, and Lung Institute
Bethesda, MD 20205

301/496-7255

Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

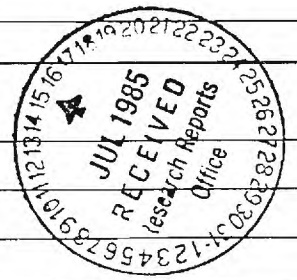
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

No Funds may be expended after 6/30/86.

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| Accounting | Research Communications (2) | Other A. Jones |

FORM OCA 65 2S5

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 9/19/86

Project No. G-33-W04 School/~~Lab~~ Chem.

Includes Subproject No.(s) N/A

Project Director(s) S. W. May GTRC / ~~GIT~~

Sponsor DHHS/PHS/NHLBI

Title Novel Antihypertensives: Rational Design and Evaluation

Effective Completion Date: 6/30/86 (Performance) _____ (Reports)

Grant/Contract Closeout Actions Remaining: Reporting will be done under G-33-W05

- [ ] None
- [XX] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Continues Project No. _____ Continued by Project No. G-33-W05

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G-33-W04

| SECTION IV<br>PROGRESS REPORT SUMMARY | GRANT NUMBER<br>HL28167 | |
|---|---|---|
| PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR<br>Sheldon W. May | PERIOD COVERED BY THIS REPORT<br>FROM | THROUGH |
| NAME OF ORGANIZATION<br>Georgia Institute of Technology | 7/1/85 | 6/30/86 |

TITLE *(Repeat title shown in item 1 on first page)*
Novel Antihypertensives: Rational Design and Evaluation

*(SEE INSTRUCTIONS)*

## PUBLICATIONS


S.R. Padgette, K. Wimalasena, H.H. Herman, S.R. Sirimannne, and S.W. May, "Olefin Oxygenation and N-Dealkylation by Dopamine Beta-Monooxygenase: Catalysis and Mechanism-Based Inhibition", Biochemistry, 24, 5826-5839 (1985).

S.R. Padgette, H.H. Herman, J.H. Han, S.H. Pollock, and S.W. May, "Antihypertensive Activities of Phenyl Aminoethyl Sulfides, A Class of Synthetic Substrates for Dopamine Beta-Monooxygenase", J. Med. Chem., 27, 1354-1357 (1984).

S.H. Pollock, H.H. Herman, M. Dillard, and S.W. May, "The Vascular and Antihypertensive Activity of Sulfur Analogs of Phenylpropylamine, Novel Substrates for Dopamine Beta-Monooxygenase", (under submission to J. Cardio. Pharm.).

K. Wimalasena and S.W. May, "Mechanistic Studies on Dopamine Beta-Monooxygenase Catalysis: N-Dealkylation and Mechanism-Based Inhibition by Benzylic Nitrogen Containing Compounds; Evidence for a Single-Electron Transfer Mechanism", (under submission to J. Amer. Chem. Soc.).

S.F. Roberts, H.H. Herman, and S.W. May, "Selenoxidation by Dopamine Beta-Monooxygenase: Product Recycling and Ascorbate Depletion", (under submission to Biochemistry).


## PROGRESS REPORT

The goals of our research program are: (1) to answer key questions regarding the biochemical mechanism responsible for the antihypertensive activity we have demonstrated for certain compounds of our design; (2) to bioassay the potential antihypertensive activity of other compounds we have already designed and synthesized based on the ideas of this proposal; and (3) to extend our efforts by continuing to design and evaluate other classes of compounds potentially capable of exhibiting antihypertensive activity through th[redacted]fication of adrenergic neuronal activity.

Substantial progress h[redacted]n all of these areas. The following paragraphs summarize our progress du[redacted]ear of the current project period.

Uptake and Enzymatic Turnover in Chromaffin Granules. The most likely mechanism by which these neurotransmitter analogs are causing an antihypertensive effect in spontaneously hypertensive rats (SHR) is thought to involve uptake into neuronal cells, followed by uptake into neurotransmitter storage vesicles. After uptake, by virtue of their ability to act either as DBM substrates or inhibitors, they effectively reduce the amount of norepinephrine (NE) stored in these vesicles and available for release and post-synaptic activation of adrenergic receptors. We have chosen initially to look at uptake into neurotransmitter storage vesicles. Since this is the critical step in the overall process, by performing these experiments first we can focus on the most promising compounds for subsequent testing in the more laborious whole-cell uptake and release experiments. Also, we are now utilizing a procedure which incorporates the recent elegant work from David Njus' laboratory where it has been demonstrated that "ghosts" derived from chromaffin granules are a much better model for these types of uptake and conversion experiments.

Using this chromaffin vesicle ghost protocol, we have now demonstrated uptake of the prototype selenium-containing analog, phenyl aminoethylselenide (PAESe), into these vesicular preparations. Furthermore, we have shown that the enzyme-dependent ascorbate recycling process we observed in free solution (see pp. 20-22 and pg. 31 of original proposal for rationale) does occur in these vesicle preps. We have demonstrated that uptake of PAESe into ghosts results in a ca. 50% reduction of vesicular ascorbate levels. In virtually identical experiments, we are now examining the effects of several of our sulfur-containing analogs on the vesicular uptake and conversion process.

We chose to begin these vesicle studies by using the selenium-containing compound, PAESe, because of the sensitivity and ease with which we are able to measure changes in ascorbate levels within these ghosts using HPLC with electrochemical detection. For the work we propose to perform with the test compounds of the other classes, we must be able to acquire radiolabelled versions of the test compounds. To this end, we have set up a radiolabelling synthetic laboratory which yields ring-tritiated compounds of high specific activity. We have, to date, tritium-labelled one test compound, phenyl ethylenediamine (PEDA, one of our N-dealkylating analogs). During the coming year, we plan to extend this work to the other classes of compounds using essentially the same protocols.

Bioassay of Antihypertensive Activity. We have developed a chronic dosing and measuring protocol for use with our olefinic and selenium-containing compounds since preliminary tests indicated that there was a 24-48 hour latency between dosing and the beginning of the antihypertensive effect. This protocol allows us to follow groups of SHR (control and drug-treated) over a two-week or more period, with dosing on a daily basis with measurement of blood pressure, heart rate, and body weight over the entire period. Using this new protocol, we have found that phenyl aminomethylethene (PAME, our prototypic olefin-containing analog) and phenyl aminoethylselenide (PAESe, a selenium-containing analog) both exhibit a marked, dose-dependent antihypertensive effect with as much as a 50% reduction in blood pressure and heart rate which begins ca. 48 hrs. after the first dose and extends for two weeks in both cases.

As an important adjunct to these studies, we remove whole hearts at the end of the dosing period, and using HPLC with electrochemical detection, are able to demonstrate a marked change in sympathetic nerve-ending catecholamines in the drug-treated animals. We have found a >50% reduction in norepinephrine and a >70% reduction in epinephrine from hearts of animals treated with PAESe which correlates with the effects on blood pressure and heart rate already noted. In the case of PAME, these effects are ca. -54% for NE and -80% for EPI. With both compounds we are also seeing a marked decrease in the weight of the hearts of those animals receiving the test compound. We think this may reflect a "blockage" of the developement of left ventricular hypertrophy which normally accompanies the etiology of hypertension, but have not conclusively proven this as yet. During the coming year we plan to further refine the bioassay using, for example, implanted osmotic pumps to continuously deliver compound doses in SHR, and plan to extend this bioassay to other compounds of different classes.